PRELIMINARY NOTE ON THE SPERMATOGENESIS OF PEDICULUS VESTIMENTI.[1]

KATHARINE FOOT.

In response to a request from Col. Alexander Lambert I came to Paris, December, 1917, in order to study *Pediculus vestimenti* from the point of view of the biologist in the hope of getting some data that might be of service to the investigators who were studying the louse as a possible transmitter of certain diseases prevalent among the troops.

In an exhaustive and masterly study of the problem in relation to trench fever, Col. Strong has shown that the lice can unquestionably transmit this disease and has proved beyond question what was heretofore merely a surmise.

Such data as I have been able to collect that may have some bearing on the problems in relation to disease have been reported to the Research Department of the American Red Cross; but I have omitted from such reports purely cytological data which have no apparent practical value.

As far as I know, no report has been published of any work done on the chromosomes of the louse, and as *P. vestimenti* has been classed as an Hemipterous insect I was interested to see if the chromosomes have the same bizarre morphological characteristics as are typical of so many Hemiptera.

The spermatocyte chromosomes are very minute, so minute that I have as yet found it impossible to demonstrate the method of division of the second spermatocytes; but I have several entirely satisfactory stages of the first spermatocyte chromosomes, and as these have the same morphological characteristics as the corresponding stages in other species of Hemiptera, it is quite permissible to assume a like correspondence for the second division.

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[1] My grateful acknowledgments are due to the late Professor Blanchard, who gave me a most cordial welcome to his laboratory at the Ecole de Médecine. He not only gave me ample space for my work but the sympathy and encouragement I received from him and the members of his staff made it possible for me to continue my investigations of these repulsive insects.

The above mentioned morphological peculiarity of the chromosomes of many Hemiptera *i. e.* the unequal bivalent—called by Wilson the X Y chromosome—is present in the first spermatocytes of *Pediculus vestimenti* and its division is typical—the two unequal parts dividing as univalents in the first division. The unequal bivalent is demonstrated in the metaphase stages of Figs. 10, 11, 12, and the division of the smaller half of the unequal bivalent is shown in Fig. 13.

Miss Strobell and I (1914) published a series of photographs showing both the first and second spermatocyte divisions in *Euschistus variolarius, Euschistus servus* and in two generations of hybrids from *E. variolarius* by *E. servus.* A comparison of these photographs of the first spermatocyte chromosomes with the above mentioned Figs. 10–13 will show that the first spermatocyte chromosomes of *Pediculus vestimenti* are of the same type as in the species of *Euschistus* referred to, and it is therefore quite logical to assume that the second spermatocyte chromosomes are equally typical though its demonstration is not yet possible.

In Fig. 16 (on the right) is a group of chromosomes from an embryo in an egg at the basal end of the ovary.

It is not possible to determine the exact number of chromosomes of this group, as 18, 19 or 20 can be counted.

The first spermatocyte chromosomes indicate that the somatic number should be ten and in the few spermatogonial groups I have found it possible to identify ten chromosomes; but they are so small and so frequently constricted that the estimate can always be questioned.

I am convinced that the most favorable stage for an exact interpretation of the louse chromosomes is the first oöcyte prophase, but to secure this stage involves a patient search which cannot be undertaken at present. Fig. 18 indicates that it will be possible to find the later prophase stages, for in this preparation the chromosomes are nearly formed, and it ought to be possible to find the slightly later stages in the same locality of the ovary.

An early stage of the yolk spheres of the ovary is shown in the photomicrograph of Fig. 16 (on the left). These are chromosome-like structures, which in the early stages select the chromatin stains and are morphologically so like chromosomes that one is tempted to interpret them as chromosomes which develop into yolk-spheres.

A series of stages prior to the first spermatocyte metaphase is shown in Figs. 1 to 9. The centrosomes of the first spermatocytes (Figs. 1 to 4) are a feature of these stages which is most marked. The position of these centrosomes in relation to the nucleus is very variable: they are in contact with the nucleus or almost in contact with the periphery of the cell or in any position between these two extremes. In later stages they are frequently divided into two or more, rarely into three, parts. Several of the former are shown in Fig. 7 (the centrosomes of this figure are a little exaggerated by the artist).

The Development of the Spermatid into the Mature Spermatozoön.—The most striking feature in the development of the spermatozoön is the duplex character of the tail. Apparently the tail is composed of two distinct and independent filaments, the apparent independence of these filaments being more marked in the earlier stage (Figs. 20–21). One would naturally suppose that one of these filaments represents the flagellum of other forms, but it has not been possible to demonstrate any substance connecting the two filaments, though the fact that even in smear preparations the two are never found widely separated would indicate that they are attached by some connecting substance.

Historical Sketch.

Certainly no insect has been accused of being the promoter of a greater variety of diseases than the louse and perhaps no accused has been charged with so many crimes on less evidence. *Pediculus* has been credited with transmitting the following diseases: typhus fever, typhoid fever, recurrent fever, trench fever, tuberculosis, spinal meningitis, plague, leprosy, beri-beri and more than a dozen minor skin diseases. In some of these cases the evidence seems to be confined to the fact that the patient may be infested with lice.

For typhus fever, recurrent fever and trench fever it has been proved that the lice do in fact transmit these diseases, but details as to the method of transmission are still disputed. It is held by many investigators that germs are not transmitted by the bite of the louse—the sole method of transmission being infection from their excrements. These are freely deposited on the skin and in the clothing of the host and subsequent scratching of the skin induced by the intense itching of the bites not only lacerates the surface but frequently causes a deep wound that leaves a scar lasting many months. It is self-evident that such lacerations over surfaces more or less infested with the excrements of the lice may cause a most effective inoculation. This method of infection by the fæces or the crushed body of the louse has been demonstrated for typhus fever by Nicolle (1909), Nicolle, Conte and Conseil (1910) and others. For recurrent fever by Sergent and Foley (1910), Sergent, Gillot and Foley (1911), Nicolle, Blaizot and Conseil (1912) and others. For trench fever, by R. P. Strong (1918). Familiarity with the feeding habits of the louse demonstrates the danger of this method of inoculation, for the amount of excrement discharged by each louse is surprising. I have frequently seen a single louse, during one hour's feeding, discharge excrement ten times, and five times is not unusual. For more than a year I have closely studied the feeding habits of *Pediculus vestimenti* and in my report to the research department of the Red Cross I described their behavior as follows: "Observations made during the feeding hour demonstrate that individual lice may behave very differently. As a rule they bite at once when young and vigorous. Some become gorged with blood in ten minutes and will not bite again, though most frequently they bite several times during the hour, moving around rather restlessly between times. Others bite continuously the entire hour, casting their excrements while biting. The old lice frequently do not bite for several minutes or even half an hour and then suck the blood very deliberately." I am inclined to believe that the method of biting demonstrated for one hour indicates the method for the entire twenty-four hours and that therefore the younger lice are almost continuously feeding on the host, wandering about and biting very frequently.[1] This would accord with observations made by Miss Strobell and

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[1] These observations support Nuttall's (1917) conclusions as to the probable feeding habits of lice. He thinks they bite very frequently, for when raising them on his wrist he noticed they started to bite at all times when he was quiet.

myself on other species of Hemiptera, *Euschistus variolarius*, *E. servus*, *E. ictericus*, etc., etc. These insects were fed on fruit and could be closely observed during the twenty-four hours. They fed almost continuously during the night as well as during the day, and this leads me to surmise that the lice may feed with equal frequency and explains the torment that soldiers suffer even when infested with relatively few lice and suggests the possibility of inoculation from a single louse.

The disputed question whether simply the bite of the louse can inoculate the host has given rise to much discussion, some investigators emphatically denying that any danger is caused by the bite alone. Colonel Strong (1918) conducted some experiments with the aim to determine this point and concludes that "it seems fair to argue that the bite is probably a common mode of infection." He states that in some instances the disease was produced by pure biting experiments. His summary of the probable methods of infection through biting is as follows:

1. By piercing or stabbing and inoculating with mouth-parts contaminated with infected material such as blood from the patient or by louse fæces and body juices.

2. By stabbing and inoculating from the skin which has been contaminated with infected material such as louse fæces, and possibly body juices.

3. By stabbing and inoculating with mouth-parts which have been contaminated with virus grown or developed in the stabber-sac.

4. By stabbing and regurgitating of the virus from the alimentary canal.

5. By stabbing and the injection of the virus contained in the salivary juices.

6. By hereditary infection.

One of the difficulties in determining the value of the bite alone is to eliminate the fæces from the experiment. Those cases in which this is assumed to be done by allowing the louse to bite through chiffon do not appear to me to be conclusive, for in my experience they will not bite unless the chiffon is pressed upon the surface with sufficient strength to force the skin through the interstices of the chiffon, in which case the only effect of the chiffon is to reduce the area of the skin exposed, and the lice wander over the exposed area distributing the fæces as usual.

The danger of the louse as a promoter of disease has been so long appreciated that he has claimed the attention of a large number of investigators, the French and English forming the majority. The work accomplished up to 1917 has been most ably presented by Professor Nuttall, of Cambridge. His bibliographical list is an index of the thoroughness of his historical study of the subject. He has listed nearly 600 investigators.

A second historical sketch was published in France the same year (Souéges et du Noyer, 1917). These two studies are a convenient record of all the historical data that can be of value to the investigator.

Two English investigators (Warburton, 1909, and Fantham, 1912) were the first to study the life history of *Pediculus* and their results were supported and extended by Bacot in 1916. He determined the number of moults to be three, the average length of life of the louse, the average number of eggs deposited daily by a single female and other details, all of which my investigations support although our methods of work differed materially. He used an entomological box containing a number of lice and strapped this box on his person each night, allowing the lice to bite from six to seven hours daily. My lice were fed only one hour in the twenty-four and in such a manner that I could watch them while feeding.

The most serious difficulty in the investigation of lice is the food supply. In all the accounts with which I am familiar the investigator has had sufficient self-abnegation to feed his lice on his own person, but not having reached those heights myself, my initial difficulty was to find a host. There seems to be something extremely ridiculous in the mere suggestion of feeding a louse, for my most serious and generous offers received the discouraging response of a broad grin and an emphatic shake of the head. I finally succeeded in securing a host at the Asile de Nuit—a night employee of that institution. He was an old sailor whose evident familiarity with *Pediculus* at the Asile de Nuit had led him to cease to regard them in a humorous light, and he proved to be a thoroughly dependable food supply. He never missed his daily hour in my laboratory for the five months I employed him.

When feeding the lice I at first used the usual method of putting a number in a tube, inverting the tube on the arm and holding it securely in place to prevent the lice from escaping. I found this method unsatisfactory for several reasons and devised therefore quite a different technique. Lice cannot crawl up a glass surface if it is clean and are therefore perfectly safe in a glass ring even if it is only 2 cm. high. I had such rings made to order and fastened them securely onto the arm with melted paraffine. In this manner several different experiments can be conducted at the same time and the generations can be kept separate—further the lice can be conveniently studied with a lens during the hour they are feeding. For the remaining twenty-three hours they were kept in a Pasteur incubator at a temperature between 27° and 29° C. While in the incubator the lice were kept in cages such as those used in the laboratory for raising various insects. This cage is the tube de Borel, in which is placed an inner tube for the insects, this being held in the center by absorbent cotton which is kept wet to insure sufficient moisture. I found the use of absorbent cotton very inconvenient and replaced it with a short tube having an aperture large enough to contain the inner tube and open at both ends with a lip at each end sufficiently wide to center it in the tube de Borel. The inner tube in which the insects are kept is dropped into this shorter tube and an inch of water kept in the tube de Borel. I found this method a great economy of time, for it was necessary to pack the cotton around the inner tube with much care, since if the opening came in contact with the inner surface of the tube de Borel (often quite wet) a drop or two sometimes dripped into the inner tube and cost the life of one or more nymphs.

Several years of experience in crossing and raising other species of Hemiptera have been my guide in raising the lice. Miss Strobell and I found that the species we studied required as much humidity as possible while avoiding any condensation of the moisture. This I have found true for lice—a half a drop of water or less can kill a nymph. If he gets on his back on the glass in even a fraction of a drop of water, he cannot regain his feet until the water dries and if the glass is not clean he adheres to it and finally dies.

After trying the usual method of keeping the lice on small pieces of woollen or muslin cloth it occurred to me that a large number of short pieces (about 8 mm.) of soft, coarse thread would have many advantages. First they would be much more sanitary for they can be changed every day if necessary without disturbing the lice at all. When lice are on a small piece of cloth, the cloth becomes filthy in a few days and it is exceedingly difficult to remove the lice to fresh pieces. Further the thread avoids all the difficulties encountered in transferring and counting the lice. They cling to a thread with great tenacity; therefore single lice can be carried on a thread any distance with perfect safety. They deposit their eggs on the thread and therefore the eggs deposited each day can be conveniently collected and isolated. Using these threads made it a simple matter to record the following life history of a single pair of lice. The pair was hatched from eggs deposited in the laboratory and had their third (final) moult August 19. They were seen mating August 22. The next day 4 eggs were deposited and thereafter 4, 5 or 6 were deposited daily until the female died.

RECORD FL. D.

1918.		Eggs.	Hatched.
Aug.	(Mated Aug. 22)		
23	Mated	4	
24		3	
25		5	
26		4	
27		4	
28		5	
29		5	
30		4	
31		5	
Sept.			
1		5	
2	Hatched the 10th day	6	1
3		5	3
4		5	4
5	(Mated)	5	3
6	(Male dead—each daily deposition of eggs kept separate from death of male)	5	7
7		5	3

1918. Sept.	(Mated Aug. 22)	Eggs.	Hatched.
8		6	5
9		5	5
10		5	5
11		6	4
12		5	5
13		5	6
14		5	4
15	Female dead	1	5
		113	
16			5
17	5 hatched from eggs deposited 9–7		6
	1 " " " " 9–8		
18	3 hatched from eggs deposited 9–8		4
	1 " " " " 9–9		
19	2 hatched from eggs deposited 9–8		6
	3 " " " " 9–9		
	1 " " " " 9–10		
20	1 hatched (the last) of eggs deposited 9–9		7
	3 " from eggs deposited 9–10		
	1 " " " " 9–11		
	2 " " " " 9–12		
21	1 hatched (the last) of eggs deposited 9–10		11
	5 " (the last) " " " 9–11		
	2 " from eggs deposited 9–12		
	3 " " " " 9–13		
22	2 hatched (the last) of eggs deposited 9–13		5
	2 " from eggs deposited 9–14		
	1 " " " " 9–15		
23	3 hatched (the last) of eggs deposited 9–14		3
	The last egg deposited 9–12 not hatched, nymph partly emerged but dead.		

SUMMARY.

If we omit the one egg deposited the day the female died the daily average for the remaining 112 eggs is 4.9.[1]

113 eggs were deposited from August 23 to September 15 and all of these developed to the nymph stage, though six failed to completely emerge from the egg.

The above record shows that the male died September 6 and the female died September 15. From September 6 each daily deposition of eggs was isolated to determine how long the eggs were fertilized after the last mating (September 5). The record shows that all the eggs developed and that all hatched but one.

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[1] Bacot found the daily average to be 5.1 and Nuttall's experiments demonstrated that under natural conditions, *i.e.*, when the lice lived continuously on the host, the daily average increased to 9.7.

As the female had her third moult between the 18th and 19th of August, she lived only 28 days after maturity, less than the average length of life for a female; but other females in the laboratory kept under the same conditions lived 42 days, 40 days, 39 days, etc. Bacot found the average length of life of a female to be 34 days. If my lice are a little below this average I think it is probably due to the difference in feeding—he fed his lice six or seven hours daily and mine were fed only one hour daily.

According to my experience, the longer a race is bred in the laboratory the less prolific they become and the death rate is much higher. I believe this is due entirely to an abnormal lack of nourishment. Feeding only one hour in twenty-four is certainly very abnormal for these insects.

One cannot study the record of the large amount of experimental work done on the louse without being impressed with the need of feeding these insects apart from the human host before certain problems now in dispute can be solved. My efforts have been largely given to this well high hopeless task which is my apology for a very superficial study of the spermatogenesis.

PAPERS REFERRED TO.

Bacot, A.

'16 Notes on *Pediculus humanus* and *Pediculus capitis*. Brit. Med. Journ.

Foot, Katharine and E. C. Strobell.

'14 The Chromosomes of *Euschistus variolarius*, *Euschistus servus* and the Hybrids of the F_1 and F_2 generations. Arch. für. Zellf., Bd. 12.

Langeron, M.

'16 Précis de Microscopie. Masson & Cie, Editeurs, Paris.

Nicolle, C.

'09 Reproduction expérimentale du typhus exanthematique chez le singe. C. R. Acad. des Sc., 149.

Nicolle, C., Conte and Conseil, E.

'10 Transmission expérimentale du typhus exanthematique par le pou du corps. Ann. Inst. Past., 24.

Nicolle, C., Blaizot, L. et Conseil, E.

'12 Etiologie de la fièvre récurrente. Son mode de transmission par le pou. C. R. Acad. des Sc., 154.

Nuttall, George.

'17 Bibliography of *Pediculus* and *Phthirus*. The Part played by *Pediculus humanus* in the Causation of Disease. Parasitology, Vol. X.

Sergent, E. and Foley, H.

'10 Recherche sur la fièvre récurrente et son mode de transmission dans une épidémie algérienne. Ann. Inst. Past., 24.

Sergent, E., Gillot, V., et Foley, H.
'11 Typhus récurrent Algérien. Sa transmission par les poux. C. R. Soc. Biol., 70.

Soueges, R. et Du Noyer, M. R.
'17 Les Poux, le mal qu'ils nous causent. Bull. des Sciences Pharmacologiques t. XXIV.

Strong, Richard P.
'18 Trench Fever. Report of Commission Medical Research Committee. American Red Cross. Oxford University Press.

EXPLANATION OF PLATES.

All the sketches were drawn at a magnification of about 750. Zeiss hom. immer. 2 mm. 140—apo. oc. IV camera lucida.

TECHNIQUE.

Smear preparations stained with May-Grünwald followed by Hollande.

These stains were used in accordance with the method published by Dr. Langeron (1916) and I am greatly indebted to him for many valuable suggestions and encouraging interest in my work.

PLATE I.

FIGS. 1, 2, 3, 4. First spermatocyte rest stages, each showing a distinct centrosome in varying proximity to the nucleus. No cell membranes are differentiated.

FIG. 5. First spermatocyte nucleus showing a single nucleolus and the chromatin segregating to form the chromosomes.

FIG. 6. Two first spermatocyte nuclei with granular chromatin segregating to form the chromosomes. The nucleolus has disappeared.

FIG. 7. Numerous first spermatocyte nuclei showing successive stages of the differentiation of the chromatin. In the earlier stages the chromatin is apparently homogeneous and later it is granular and segregating into definite masses to form the chromosomes. A few centrosomes are differentiated.

FIG. 8. Numerous first spermatocyte nuclei showing later stages than those of Fig. 7. In many of the nuclei the chromatin has segregated into 5 distinct masses foreshadowing the 5 bivalent chromosomes of the first spermatocyte metaphase.

FIG. 9. First spermatocyte nuclei about the same stage of development as those of Fig. 7.

FIGS. 10, 11, 12 and 13. Each figure shows the 5 bivalent chromosomes of the first metaphase. In each figure one or more of the chromosomes is a dyad, foreshadowing the first division. An unequal bivalent, which is typical of so many Hemiptera, is clearly shown in each group.

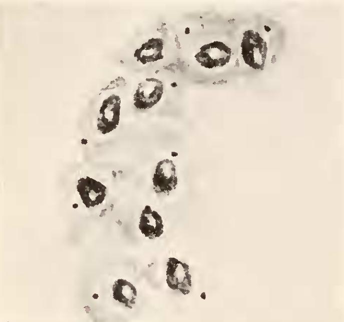
FIG. 13. All the chromosomes of this first metaphase group are dyads, foreshadowing the division of each. The large and small chromosomes of the unequal bivalent are detached and each is a dyad. This indicates that each will divide in the first division and that therefore the resulting halves will undoubtedly separate in the second division in the manner typical of so many Hemiptera.

FIG. 14. Late anaphase of the second division. The chromosomes are too small and too closely segregated to determine their number and form.

FIG. 15. Three telophases of the second division. Each shows an unequal division of the chromatin, this being the sole evidence, at this stage, of the separation of the large and small moieties of the unequal bivalent.

FIG. 16. On the left a photomicrograph of a small group of chromosome-like structures from an immature ovary. From these the yolk-spheres are developed. × 450. On the right a sketch of a group of chromosomes from an embryo in an egg at the basal end of the ovary.

FIGS. 17, 18. Two germinal vesicles from young ovarian eggs. In Fig. 17 the chromatin has partly segregated into threads, and a single nucleolus is present. Fig. 18 shows numerous small dense nucleoli, and the separate chromatin threads suggest a progressive step in the forming of the chromosomes though their abnormal number may be due in part to the technique.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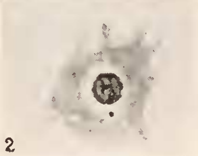
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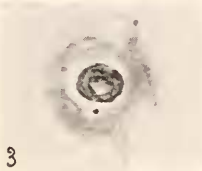
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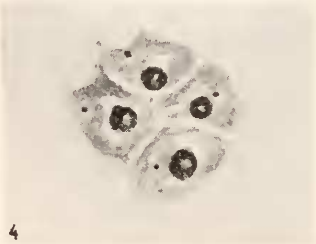
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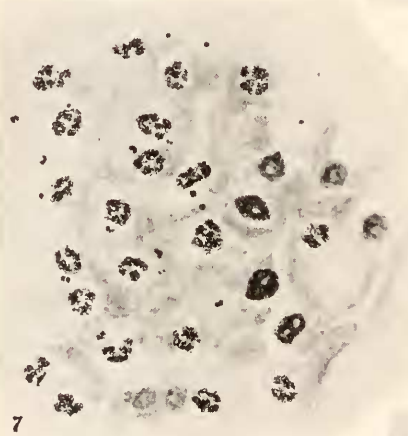
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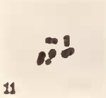
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18

KATHARINE FOOT.

EXPLANATION OF PLATE II.

FIG. 19. Numerous heads of spermatids shortly after the second division. They are at first round, as demonstrated in the figure, and, as a rule, elongate before the appearance of the tail. In the elongated heads of this figure the middlepiece (?) is in evidence and from this point the tail develops.

FIGS. 20 to 27. Successive stages of the development of the spermatid into the mature spermatozoön. In Figs. 20 and 21 the middlepiece is demonstrated, the tail developing from this point. In Figs. 22 and 24 both the middlepiece and the acrosome (?) are demonstrated, the spine developing from the latter. Fig. 25 shows an early stage of the development of the spine. Figs. 26 and 27 demonstrate the mature spermatozoön in which the head and spine are fully developed. At this stage the middlepiece and acrosome are obscure.

19.

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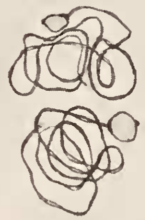
21

22

23



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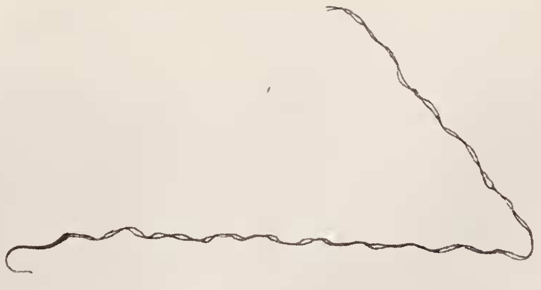

27

DETERMINATION OF THE SEX OF THE OFFSPRING FROM A SINGLE PAIR OF PEDICULUS VESTIMENTI.

KATHARINE FOOT.

Nuttall (1917) gives a summary of the data as to the proportion of the sexes in *Pediculus vestimenti*. He says "the proportion of the sexes as determined by raising experiments has yielded contradictory results," and this he thinks is due to the small number of the broods of the experiments. In his own experiments of mixed pairs he found the sexes nearly equal, though his broods also were small.

Hindle (1917) discovered a marked inequality in the proportion of the sexes of certain pairs, and he concludes this to be the normal condition, basing this conclusion on the determination of the sex of 25 single pairs.

It has been proved by the observations of several investigators that a single female may deposit from one hundred to nearly two hundred eggs. It is obvious, therefore, that the sex must be determined for at least one hundred of a generation or the results are inconclusive. Certainly at least 75 per cent. of the eggs deposited must hatch and the sex be determined for these nymphs.

Hindle secured such a small number of individuals from each of his 25 pairs that Nuttall seems more than justified in questioning his conclusions. The number of individuals in each of the 25 broods studied by Hindle is as follows: 2, 4, 6, 9, 8, 10, 11, 12, 15, 17, 24, 25, 26, 26, 27, 29, 30, 31, 32, 34, 36, 38, 45, 48, 64. Fifteen of these 25 broods were all males or all females, *e.g.*, the brood of two were both females, the brood of four were also females, one of the broods of nine were males and the other brood of nine were both males and females. The brood of sixty-four were all females but as they probably do not represent more than 50 per cent. of the offspring from that pair, the evidence, even in this case, is without force.

METHODS AND RESULTS.

In order to determine the sex of as large a number of the offspring as possible I adopted a different method from that of other investigators. Instead of waiting for the nymphs to mature in order to determine the sex, they were dissected at any stage that was convenient and those that died were not discarded but dissected at once and the sex recorded. It was thus possible to determine the sex of nearly all that were hatched.

Our method of treating these insects makes it possible to collect and isolate the eggs deposited each day, thus keeping an accurate count of the number of eggs deposited daily and the number hatched.

Both the male and the female had the third moult in the laboratory, the female July 22, 1919, and the male July 24.

The male died August 26; he lived therefore 33 days after maturity, thus exceeding the average length of life of *P. vestimenti* males. The female died September 5. She lived therefore 45 days after maturity, eleven days longer than the average length of life of the females. She continued to deposit eggs daily after the death of the male. She deposited in all 143 eggs (116 before the death of the male and 27 after that date). She commenced to lay eggs the 4th day after maturity and stopped laying 2 days before she died. She deposited 2, 3, 4 or 5 eggs each day. Of the 116 eggs laid before the male died, 11 failed to hatch, but nearly all developed and three of the nymphs partly emerged. Of the 27 eggs laid after the male died, seven failed to hatch, but all were fertilized and four of the nymphs partly emerged.

The nymphs were raised in lots of 20, and the death rate was relatively low in lots 1, 2, 3 and 4. The death rate in lots 5 and 6 was much higher. Several of the nymphs died even before the first moult. In some of these cases the nymphs were too dry to dissect, and therefore the sex was undetermined. This higher death rate was probably due to the age of the female, as the death rate was progressively high towards the end of the experiment.

125 eggs were hatched and the sex was determined for 115 (62 males and 53 females). 42 died during the experiment and

these were dissected as stated above and their sex determined. Of these 27 were males and 15 females.

For the first half of the experiment the females were more numerous than the males; the proportion of males then gradually increased until the final result gave 62 males and 53 females.

These results are of value only because the sex was determined for 92 per cent. of the entire generation, but the experiment must be repeated many times before the evidence can be of scientific value.

PHYSIOLOGICAL STUDIES ON PLANARIA.

III. OXYGEN CONSUMPTION IN RELATION TO AGE (SIZE) DIFFERENCES.

L. H. HYMAN,

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I. Review of Literature.

The present paper is a report of some determinations of the rate of oxygen consumption of small (young) as compared with large (old) planarians belonging to three different species. That the metabolic and other activities of small individuals are more rapid than those of large individuals of the same and different species is so well known from a number of independent investigations that further evidence appears almost superfluous. The majority of these investigations, however, deal with vertebrates, where a certain difficulty inherent in the nature of the material is encountered. This difficulty centers about the impossibility of finding a proper unit for comparison of animals of different sizes. Among the warm-blooded vertebrates, the metabolic rate has commonly been reckoned per unit of surface area on the necessity of taking into account loss of heat by radiation. But leaving out of consideration the difficulties of accurately measuring the surface, over which proceeding a controversy of long standing exists, the surface method of comparison is more or less meaningless in the case of cold-blooded animals. Nor is the method of comparison of metabolic rate per unit weight free from objection. A considerable part of the weight of the vertebrates and the higher invertebrates consists of skeletal material, connective tissue, fat, etc. The metabolic rate of such material must be relatively low, and further its proportion to the total weight must be different in animals of different sizes; nevertheless, these factors have not been considered in any of the researches on the subject, nor, as far as I am aware, has anyone attempted to make a correction for these inert portions of the body.

As Benedict and Talbot ('14) concluded after a study of the metabolism of infants, metabolism cannot be accurately measured either by weight or by surface, but only by the amount of active protoplasm. It is probably impossible to determine this but certainly in the soft-bodied lower invertebrates, weight is a more accurate index of the amount of active protoplasm than is the case in higher forms, where skeleton is present. For this reason, the metabolism of the lower forms deserves more attention than it has hitherto received from physiologists.

Among the early investigators of the effect of size differences on metabolic rate were Jolyet and Regnard ('77), who determined the rate of oxygen consumption per unit weight of a large number of fish, and some of the higher invertebrates. They found that among related groups small species consume more oxygen per unit weight per unit time than large species; and small individuals more than large individuals of the same species. Vernon ('95) confirmed this conclusion. He measured the rate of oxygen consumption per unit weight of large and small individuals of the same species, using as material a hydrozoan medusa, a scyphozoan medusa, two ctenophores, two gasteropods, and two pelagic tunicates. With a few exceptions the smaller individuals were found to consume relatively more oxygen than the large ones. Bounhiol ('02) working with twelve species of polychæte annelids, belonging to several different families, found that the carbon dioxide production was greater the smaller the individual. This was true for individuals of the same and of different species. On the other hand, Montuori ('13) was unable to come to any definite conclusion from his measurements of the oxygen consumption of a large number of species belonging to most of the aquatic groups. In some cases the small individuals respired relatively faster than large ones of the same species; in others the reverse was found; and in still others there was no relation between size and rate of respiration. The great irregularity of Montuori's results, as well as their disagreement with the work of others, suggests that he failed to control adequately the conditions under which the experiments were performed. Child

('19) found that small individuals of *Planaria dorotocephala* produce more carbon dioxide per unit weight than large ones, and this experiment is now regularly performed as a class experiment. Allen ('19) has shown that the rate of oxygen consumption of *Planaria agilis* per unit weight is greater the smaller the animals, and reports that Miss Wolf working in the same laboratory found the same to be true for the leech, crayfish, branchipus, may-fly nymph, and stone-fly nymph. It is also the case with dragon-fly nymphs, as shown by Mr. G. C. Hawk in this laboratory. According to Morgulis ('15), the oxygen consumed per gram per hour by flounders is in general greater for small than for large animals. Tashiro and Adams ('14) note that the carbon-dioxide production of the ganglionic cord of the heart of *Limulus* is relatively greater in small than in large cords. Nicolas ('18) found that the young leaves and stems of plants give off from 3½ to 7 times as much carbon-dioxide per gram per hour as old leaves and stems from the same branch.

A considerable amount of labor has been devoted to this problem in the case of mammals, especially man. Rubner originally maintained that warm-blooded animals of different sizes produce the same amount of heat per unit surface, but subsequent investigations have shown that this point of view is erroneous. Thus Magnus-Levy and Falk ('99) clearly showed that the oxygen consumption and carbon-dioxide production is highest in children and decreases with age, as measured either per unit weight or per unit surface. If individuals of the same size and weight but of different ages are compared, the younger ones respire the faster. The authors conclude that the protoplasm of children has a definitely higher rate of respiration per unit weight than that of mature individuals. References to other work supporting these conclusions will be found in their paper. Recently Du Bois ('16) and Gephart and Du Bois ('16) have verified this earlier work. The graphs and tables presented by Du Bois show that the heat production as measured per kilogram of weight is highest in infants and decreases gradually; as measured per unit of surface, it is low in infants, increases rapidly during the first year, reaches a maximum between the ages of 1 and 6, falls rapidly to 20, and thereafter decreases more slowly. According to Benedict and Talbot ('14, '15), the metabolism of infants is about the same as that of adults, but the results were very variable. It seems highly probable that the metabolism of infants cannot properly be compared with that at other ages because: (1), the heat-regulating mechanisms of infants are known to be very imperfect; (2), infants commonly have relatively more fat than is present in normal individuals of other ages; and (3), the muscle tone of the voluntary muscles of infants must be lower than it is at other ages. Since a large part of the heat production of mammals originates in the voluntary muscles, this difference in muscle tone alone makes impossible any real comparison of the metabolism of infants with that of later stages of ontogeny when the muscles are in full use.

A few researches have been carried out on mammals other than man. Thus Slowtzoff ('03) working on dogs found that the oxygen consumption and carbon dioxide production per kilogram per minute is greater in small than in large individuals. A. V. and A. M. Hill ('13) determined the same relation in rats. Among birds, Bohr and Hasselbalch ('00) observed that the carbon dioxide production per kilogram per hour is considerably higher in newly hatched chicks than in the adult hen.

These researches are sufficiently numerous to establish the generalization that smaller (younger) individuals have a higher metabolic rate than larger (older) ones. The investigations on man clearly show that the difference is due to age and not to size. Age is also probably the determining factor in the metabolic difference found between large and small species, since, in general, species which grow to a large size must be older by the time they have attained that size than are adult individuals of small species.

How far back in the ontogeny can this generalization be carried? At what point in the ontogeny does the metabolic rate attain its highest value? Few researches have been carried out upon these points. The eggs of animals are probably cells of very low metabolic rate. After fertilization, the metabolic rate gradually rises, as development proceeds, up to a certain point and then falls. In the sea-urchin egg (*Arbacia punctulata*), the oxygen consumption was found to rise continuously up through late cleavage (determinations stopped at this point); and in the eggs of *Fundulus heteroclitus*, up to the time when the embryonic axis is established after which it fell, rising again later apparently as the result of functional activity (unpublished personal observations). Unfortunately in these cases, nothing is known of the metabolic rate of the adult. Hasselbalch ('00) and Bohr and Hasselbalch ('00) have measured the rate of carbon dioxide production and oxygen consumption per kilogram in chick embryos. Both were found to be very high in the earliest stages determined—five or six days,—much higher than those of the adult; the rates then fell rapidly. The carbon dioxide output fell to the ninth day, after which it was about contant, and about equal to that of the adult. The oxygen consumption fell to the eleventh day, then rose again, and on the sixteenth day was again considerably higher than that of the adult. Bohr ('00) compared the carbon-dioxide output of guinea-pig embryos with that of the mother. He did this by cutting open the uterus under anæsthesia, determining the carbon-dioxide output of the mother and fœtuses, then clamping the umbilical cords of the fœtuses, and again measuring the CO_2 output of the mother. The difference between the two measurements supposedly represents the carbon-dioxide production due to the embryos. Of the six experiments of this kind performed by Bohr, five showed the carbon-dioxide production of the fœtuses to be greater than that of the mother. The case where the youngest embryos (5.5 grs. each) were encountered gave a carbon-dioxide output very much higher than that of the mother; the next size (16.5 grs. each) gave less CO_2, but still at a rate considerably higher than that of the mother; while in the other four cases, with large fœtuses, the CO_2 production was slightly higher than that of the mother in three, lower in one.[1]

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[1] I wish to state that the conclusions which I have drawn after careful perusal of the papers of Bohr and Hasselbalch are somewhat different from those stated by the authors. They conclude, curiously enough, that the respiratory rate of the embryos of the hen and guinea pig is no higher than that of the adult, and anyone reading their conclusion alone would certainly be misled. The authors have either overlooked or ignored the fact, which their experimental data clearly show,

These researches indicate that the rate of respiratory exchange is very high at some certain stage in the embryonic development, this stage probably differing in different animals, and falls subsequently. Later it probably rises with increased functional activity. It certainly seems to me that the metabolic rate of embryos, especially vertebrate embryos, cannot vaildly be compared with that of post-embryonic stages, owing to the enormous differences in functional activity which exist between two such stages. The comparison of the metabolic rate of a chick embryo with an adult hen seems to me a simple absurdity when one reflects upon the difference in muscle tension alone at the two stages. The fact that the total metabolic rate of chick embryos was at no stage found to be less than that of the hen, certainly indicates that if cells of the same degree of functional activity could be compared in embryo and adult, the metabolic rate of the embryonic cells would be vastly the greater. The same criticism applies to cases where the young remain more or less helpless after birth or hatching.

In addition to these researches in which direct measurement of the rate of oxygen consumption or carbon dioxide output of young and old animals have been made, a considerable mass of data is available in which another method was employed. This is the direct susceptibility method extensively used in this laboratory by Child and others; it consists in observing the time of death of organisms in lethal solutions of various substances. We have brought forward a large amount of evidence[1] to indicate that the time of death in such solutions is an index of metabolic rate, individuals of higher metabolic rate dying first. When individuals of different ages are compared by this method, it is invariably found that the time of death is shorter the younger the individual, *always providing that the same degree of functional activity is present in the animals which are being*

that the rate of respiratory exchange in the youngest embryos with which they dealt very greatly exceeds that of the adult. It is only at certain later stages that the rates of the two are approximately equal. The authors seem to have an idea that the rate of respiratory exchange ought to be the same throughout development, whereas their own and other data show that it is high in early stages and declines as development proceeds. This decline is probably of the same nature as that which organisms undergo from birth to maturity.

[1] A general résumé of this evidence will be found in Child, '13.

compared. This age difference in susceptibility to toxic solutions has been observed in *Paramecium*, three species of *Hydra*, a number of colonial hydroids and hydromedusæ, several species of planarians, and several small aquatic oligochætes; and the rise in metabolic rate which is a feature of early development has also been demonstrated by this method.

Not only does the rate of respiratory exchange in organisms vary inversely with age but many physiological activities exhibit the same relation. Bert ('70) was among the first to observe this fact. He noted that the rate of respiratory movements is faster in small than in large individuals of the same and related species. Ducceschi ('03) studied the rate of movement of the fins, tail, fin membrane, and operculum of a large number of fish of different sizes; the rate of movement of the maxillipeds, abdominal appendages, and claws of several crustacea; and of the fins and mantle of some cephalopods; and found that it varies inversely as the size of the animal. Mayer ('06) and Child ('18) observed that the rate of contraction of the bells of medusæ is faster the smaller the animal. The number of respiratory movements per minute of *Octopus* is greater the smaller the individual (Polimanti, '13). In four species of sea-cucumber, Crozier ('16) observed that the pulsation of the cloaca is more rapid the smaller the animal. Hecht ('16, '18) noted that small Ascidia pass relatively more water through their siphons than large ones; and further that the rate of the heart beat in Ascidia varies inversely as the size of the animal. In man, of course, it is well known that the rate of the heart beat is fastest in the fœtus and decreases progressively with age. The rate of growth follows a similar law.

The results of the present experiments are in full accord with those of previous investigators. Small (young) planarians whether asexually or sexually produced, consume more oxygen per unit weight per unit time than large (old) ones.

II. Experiments with *Planaria dorotocephala*.

Planaria dorotocephala lives in spring-fed marshes in morainic regions near Chicago. It has never been found sexually mature in nature (although sexual maturity has been experimentally induced by Dr. Child in this laboratory), but maintains itself by means of asexual fission. In fission, the posterior portion of the body pulls away from the anterior end, regenerates a head and becomes a complete small worm. Such small worms correspond in all tests which have been made upon them, to young worms in species reproducing sexually.

Six experiments were performed in which the rate of oxygen consumption of such small worms, under 10 mm. in length, was compared with that of large worms, over 20 mm. in length. In all cases, both large and small individuals were taken from the same stock and had been kept under the same conditions previous to the experiment. No worms showing signs of recent fission or regeneration were used. The heads were cut off the day before the test was made in order to eliminate movement. Two successive determinations of the oxygen consumption of each lot of worms were usually made, the worms were then weighed, and the oxygen consumption per two hours per 0.5 grams weight then calculated.

The method of determining the rate of oxygen consumption and the method of weighing have been described in previous papers (Hyman, '19*a*, '19*b*).

The results of the six experiments are given in Table I. In all cases the smaller worms consume more oxygen per unit weight per unit time than the larger ones. The per cent. difference ranges from 18 to 55, and closer inspection of the table reveals that the amount of difference is correlated with the length of time which has elapsed since the last feeding. In experiment 4, where the small worms respire but 18 per cent. more than the large ones, only two days had elapsed since feeding, while in the other experiments, where three or four days had passed since feeding, the difference is greater. As shown in a preceding paper (Hyman, '19*b*), feeding greatly increases the rate of oxygen consumption by stimulating the digestive tract. Since the digestive tract is more extensive in large than in small individuals, the difference between such individuals is decreased by feeding. As I did not perform any experiments with worms deprived of food for longer than four days, I do not know whether further starvation would increase the difference between small and large

TABLE I.

COMPARISON OF THE RATE OF OXYGEN CONSUMPTION OF LARGE (OVER 20 MM.) AND SMALL (UNDER 10 MM.) INDIVIDUALS OF *Planaria dorotocephala.*

Size of Worm.	C.c. Oxygen Consumed in 2 Hours.	Weight in Grams.	Oxygen Consumed by 0.5 Grs. in 2 Hours.	Per Cent. Difference.
Exp. 1. Worms Collected March 12, Last Fed April 2, Tested April 5. Temp. 22.5° C.				
Large	0.18 0.20	0.316	0.30	
Small	0.24 0.29	0.325	0.40	33
Exp. 2. Worms Collected March 12, Last Fed March 14, Tested March 17. Temp. 22° C.				
Large	0.12 0.11	0.187	0.30	
Small	0.23 0.17	0.255	0.39	30
Exp. 3. Worms Collected Early Winter, Last Fed April 23, Tested April 26. Temp. 21° C.				
Large	0.09 0.08	0.179	0.23	
Small	0.14 0.13	0.216	0.31	48
Exp. 4. Worms Collected March 12, Last Fed April 30, Tested May 2. Temp. 21° C.				
Large	0.16 0.17	0.160	0.51	
Small	0.22 0.24	0.192	0.59	18
Exp. 5. Worms from Mixed Stock, Last Fed May 6, Tested May 10. Temp. 21° C.				
Large	0.13 0.12	0.146	0.42	
Small	0.17 0.15	0.123	0.65	55
Exp. 6. Worms Collected May 28, Last Fed June 6, Tested June 10. Temp. 22° C.				
Large	0.33	0.370	0.22	
Small	0.33	0.272	0.31	41

worms, but it is scarcely likely since the effect of feeding has almost completely disappeared in four days. In Allen's experiments (Allen, '19) with *Planaria agilis*, worms starved 27 days were used; and in that case the difference between the largest and smallest worms was about 40 per cent. (Table I., decapitated worms).

III. Experiments with *Planaria velata*.

Planaria velata lives in the Chicago region in temporary ponds, usually those that are passing into prairie, known to ecologists as "prairie ponds." Like the preceding species it is never found sexually mature, and reproduces exclusively by a peculiar asexual method. After the worms have attained a certain size, pieces drop off from the posterior end, surround themselves with mucus, and pass into an encysted condition. This process continues until the entire worm has formed a series of cysts. Within the cysts, the pieces undergo regeneration into complete worms of very small size, and these emerge from the cysts in about four weeks. This cycle is repeated as long as the ponds contain water; after the water dries up, the cysts remain quiescent until the following spring.

Table II.

Comparison of the Rate of Oxygen Consumption of Large and Small Individuals of *Planaria velata*.

	C.c. Oxygen Consumed in 2 Hours.	Weight in Grams.	Oxygen Consumed by 0.5 Grs. in 2 Hours.
Three Lots of Worms 10–12 mm. Long, before Encystment; Collected March 7, Last Fed March 10, Tested 13. Temp. 22° C.			
Lot *C*	0.42 0.52	0.745	0.31
Lot *D*	0.58 0.52	0.774	0.35
Lot *E*	0.41	0.593	0.34
Three Lots of Worms less than 4 mm. Long, Emerged during May from Cysts Formed by Above Lots of Worms; Fed Several Times after Emergence; Last Feeding June 6, Tested June 9. Temp. 22° C.			
Lot *C*	0.16 0.16	0.125	0.64
Lot *D*	0.16 0.16	0.127	0.63
Lot *E*	0.10 0.09	0.070	0.67

Owing to the nature of the life cycle of this animal, it was not possible to test the large and small worms simultaneously but the large worms were tested before encystment, the small ones after emerging from the cysts. Three lots of each size were used. The worms were all taken from the same stock. The results are given in Table II. The heads were not removed in these cases, since the worms were also used for another experiment.

The table shows that the small worms consume 100 per cent. more oxygen than the large worms. The greater difference in this case than with the preceding species is probably due to the greater reorganization involved in the production of young worms with this species. As shown in another paper (Hyman, '19*c*), the process of regeneration of itself brings about a great increase in the rate of oxygen consumption.

IV. Experiments with *Planaria maculata*.

The so-called species *Planaria maculata* lives in the eastern United States under stones in ponds and in the Chicago region on submersed vegetation. It is highly probable that these two are not the same species as *Planaria maculata* from the Chicago region has never been found sexually mature, while that from Massachusetts is sexually mature and lays capsules all summer long; further the behavior of the two in regeneration is quite distinct. The forms used in this experiment were collected from a pond at Falmouth, Mass., and the experiments were performed at the Marine Biological Laboratory, Woods Hole, Mass., I am indebted to the director, Professor F. R. Lillie, for a research room in this laboratory.

Sexually mature worms, young worms, and egg capsules were collected at Falmouth and brought to Woods Hole. The rate of oxygen consumption of the mature worms was tested with one exception soon after they were collected. The young worms collected, together with those which subsequently emerged from the capsules, were kept for some time and fed at short intervals on liver (mostly fish liver) until a considerable number of them were at hand, whereupon their rate of oxygen consumption was determined. In all cases the heads were removed at least several hours before the test.

The results are recorded in Table III. The young worms consume about 50 per cent. more oxygen than the sexually mature individuals.

TABLE III.

COMPARISON OF THE RATE OF OXYGEN CONSUMPTION OF SEXUALLY MATURE INDIVIDUALS (15 MM. OR LONGER) WITH THE SEXUALLY PRODUCED YOUNG (5 MM. OR LESS) OF *Planaria maculata*.

No. of Lot.	C.c. Oxygen Consumed in Test.	Weight in Grams.	Oxygen Consumed, by 0.5 Grams in 2 Hours.
Four Lots of Sexually Mature Individuals, Collected July 10; First Three Lots Tested July 11; Fourth Lot Kept Until August 11, with Frequent Feedings, Last Feeding August 8, Tested August 11. Temp. 20° C.			
Lot 1....................	0.30 in 2 0.31 hours	0.700	0.21
Lot 2....................	0.32 0.32	0.677	0.23
Lot 3....................	0.30 0.26	0.680	0.20
Lot 4....................	0.09 0.07	0.152	0.17
Three Lots of Sexually Produced Young; Young and Capsules Collected July 10 Fed at Frequent Intervals; Last Feeding of Lots 1 and 2, July 28, Tested July 31; Lot 3, Last Feeding August 8, Tested August 11. Temp. 20° C.			
Lot 1....................	0.14 in 3 0.12 hours	0.101	0.32
Lot 2....................	0.11 0.11	0.091	0.32
Lot 3....................	0.16 0.18	0.117	0.28

V. CONCLUSIONS.

These experiments show that small or young planarians consume oxygen at a faster rate per unit weight than larger or older ones. As already stated, the carbon dioxide production is also inversely proportional to the size of the worms. It is true that experiments of this kind do not and cannot prove that the protoplasm of young animals actually has an intrinsically higher metabolic rate than that of older ones, for the reason that it is impossible to discover what part of the weight of an animal is active protoplasm and what part inert material. Nevertheless there cannot be any reasonable doubt that the metabolic rate is inversely proportional to age. It would be difficult to suggest any other explanation for many of the facts cited in this paper. namely, for the faster rate of respiration, faster heart beat, and more rapid rate of other physiological activities of young as compared with older organisms. The fact further that the susceptibility of young animals to a number of toxic substances is greater than that of old could scarcely be supposed to be due to a greater percentage of inert materials in the older individuals,

An interesting point brought out in these experiments is that the difference between the asexual and the sexual young and their respective adults is of about the same magnitude, when considered the same length of time after feeding. Worms produced by fission are therefore as truly "young" as those which develop from the egg.

In previous papers of this series (Hyman, '19*b*, '19*c*), it was shown that planarians which have been starved seven or eight weeks and pieces of planaria which have undergone regeneration have a much higher rate of oxygen consumption than ordinary fed worms, all tests being made, of course, a few days after feeding. Starved, regenerated and young worms therefore have this physiological characteristic in common: their metabolic rate is higher than that of large fed worms. That of starved ones is highest, regenerated ones next, and young, when produced from the egg or simple fission, least. It therefore appears that the metabolic rate of reduced forms depends primarily upon the amount of reorganization involved in their production, and is proportional to the degree of reorganization which has taken place. As a further illustration of this may be cited the much higher metabolic rate of the asexual young of *Planaria velata* than those of *P. dorotocephala*, presumably because much more extensive changes are involved in giving rise to the former. The evidence presented in these papers clearly supports the view which has been long maintained by Child—that such reorganizations due to whatever cause are rejuvenating transformations, restoring the organism to a physiological condition resembling that of the young.

V. SUMMARY.

1. The young of *Planaria dorotocephala* produced by simple fission were found to consume 15 to 55 per cent. more oxygen than large worms, the difference depending upon the length of time which had elapsed between the last feeding and the time of testing.

2. The young of *Planaria velata*, produced by an asexual process involving regeneration and a high degree of reorganization of the body, consume about 100 per cent. more oxygen than the worms from which they come. The greater difference in this species is undoubtedly associated with the method by which the young are produced.

3. The sexually produced young of *Planaria maculata* consume about 50 per cent. more oxygen than the sexually mature worms. There is thus no significant difference between sexual and asexual young, when the latter arise by ordinary simple fission.

4. This result, that young worms have a higher metabolic rate than old ones, is in accord with a considerable body of literature on other forms leading to the same conclusion; and confirms the work previously done in this laboratory upon this same point by other methods.

Allen, G. D.

'19 Quantitative Studies on the Rate of Respiratory Metabolism in Planaria. II. The Rate of Oxygen Consumption during Starvation, Feeding, Growth, and Regeneration in Relation to the Method of Susceptibility to Potassium Cyanide as a Measure of Rate of Metabolism. Am. Journ. Physiol., Vol. 49, pp. 420–474.

Bert, P.

'70 Leçons sur la physiologie comparée de la respiration. Paris.

Benedict, F. G., and Talbot, F. B.

'14 The Gaseous Metabolism of Infants. Carn. Instit. Wash. Publ. no. 201, 168 pp.

'15 The Physiology of the New-born Infant. Ibid., no. 233, 126 pp.

Bohr, Chr.

'00 Der respiratorische Stoffwechsel des Säugethierembryo. Skand. Arch. f. Physiol., Vol. 10, pp. 412–433.

Bohr, Chr., and Hasselbalch, K. A.

'00 Ueber die Kohlensäureproduction des Hühnerembryos. Skand. Arch. f. Physiol., Vol. 10, pp. 149–173.

Bounhiol, J.

'02 Recherches biologiques expérimentales sur la respiration des Annélides polychètes. Ann. d. Sci. Nat. Zool., Vol. 16, pp. 1–128.

Child, C. M.

'13 Studies on the Dynamics of Morphogenesis and Inheritance in Experimental Reproduction. V. The Relation Between Resistance to Depressing Agents and Rate of Metabolism in *Planaria dorotocephala* and its Value as a Method of Investigation. Journ. Exp. Zoöl., Vol. 14, pp. 153–206.

'18 Physiological Senescence in *Hydromedusæ*. BIOL BULL., Vol. 34, pp. 49–63.

'19 A Comparative Study of Carbon Dioxide Production during Starvation in Planaria. Am. Journ. Physiol., Vol. 48, pp. 231–257.

Crozier, W. J.

'16 The Rhythmic Pulsation of the Cloaca of Holothurians. Journ. Exp. Zoöl., Vol. 20, pp. 297–356.

Du Bois, E. F.

'16 Clinical Calorimetry. XII. The Metabolism of Boys' Twelve and Thirteen Years Old compared with the Metabolism at Other Ages. Archives Int. Med., Vol. 17, pp. 887–901.

Ducceschi, V.

'03 Una legge del movimento animale. Zeitsch. f. allg. Physiol., Vol. 2, pp. 482–501.

Gephart, F. C., and Du Bois, E. F.

'16 Clinical Calorimetry. XIII. The Basal Metabolism of Normal Adults with Special Reference to Surface Area. Archives Int. Med., Vol. 17, pp. 902–914.

Hasselbalch K. A.

'00 Ueber den respiratorischen Stoffwechsel des Hühnerembryos. Skand. Arch. Physiol., Vol. 10, pp. 353–402.

Hecht, S.

'16 The Water Current Produced by *Ascidia atra* Lesueur. Journ. Exp. Zoöl., Vol. 20, pp. 429–434.

'18 The Physiology of *Ascidia atra* Lesueur. III. The Blood System. Am. Journ. Physiol., Vol. 45, pp. 157–187.

Hill, A. V. and A. M.

'13 Calorimetrical Experiments on Warm-blooded Animals. Journ. of Physiol., Vol. 46, pp. 81–103.

Hyman, L. H.

'19a On the Action of Certain Substances on Oxygen Consumption. II. Action of Potassium Cyanide on Planaria. Am. Journ. Physiol., Vol. 48, pp. 340–371.

'19b Physiological Studies on Planaria. I. Oxygen Consumption in Relation to Feeding and Starvation. Am. Journ. Physiol., Vol. 49, pp. 377–402.

'19c Idem. II. Oxygen Consumption in Relation to Regeneration. Ibid., Vol. 50, pp. 67–81.

Jolyet, F., and Regnard, P.

'77 Recherches physiologiques sur la respiration des animaux aquatiques. Arch. d. Physiol., 2d Ser., Vol. 4, pp. 44, 584.

Magnus-Levy, A., and Falk, E.

'99 Der Lungengaswechsel des Menschen in der verschiedenen Altersstufen. Arch. f. Physiol., Suppl. Bund., pp. 314–381.

Mayer, A. G.

'06 Rhythmical Pulsations in *Scyphomedusæ*. Carn. Instit. Wash. Publ. no. 47, 62 pp.

Montuori, A.

'13 Les processus oxydatifs chez les animaux marins en rapport avec la loi de superficie. Arch. Ital. d. Biol., Vol. 59, pp. 213–234.

Morgulis, S.

'15 The Body Surface of Flounders and its Relation to the Gaseous Metabolism. Am. Journ. Physiol., Vol. 36, pp. 207–216.

Nicolas, M. G.

'18 Contribution à l'étude des variations de la respiration des végétaux avec l'âge. Rev. gen. d. Bot., Vol. 30, pp. 209–225.

Polimanti, O.

'13 Sui rapporti fra peso del corpo e ritmo respiratorio in *Octopus vulgaris* Lam. Zeitschr. f. allg. Physiol., Vol. 15, pp. 449–455.

Slowtsoff, B.

'03 Ueber die Beziehungen zwischen Körpergrösse und Stoffverbrauch der Hunde bei Ruhe und Arbeit. Arch. f. d. ges. Physiol., Vol. 95, pp. 158–191.

Tashiro, S., and Adams, H. S.

'14 Comparison of the Carbon Dioxide Output of Nerve Fibers and Ganglia in *Limulus*. Journ. Biol. Chem., Vol. 18, pp. 329–334.

Vernon, H. M.

'95 The Respiratory Exchange of the Lower Invertebrates. Journ. of Physiol., Vol. 19, pp. 18–70.

ON THE ACTION OF CERTAIN SUBSTANCES ON OXYGEN CONSUMPTION.

III. ACTION OF POTASSIUM CYANIDE ON SOME CŒLENTERATES AND ANNELIDS.

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In this paper are presented some further data concerning the effect of potassium cyanide on the rate of oxygen consumption of animals. In view of the importance of this substance as a reagent in physiological experiments, and since tests of this kind have been made upon comparatively few animals, it has seemed worth while to obtain data upon some representatives of groups in which the action of cyanide has not yet been determined. No experiments have, to my knowledge, been performed upon annelids, and only one cœlenterate has been tested, namely *Gonionemus*, in which form Loeb and Wasteneys ('13) showed that potassium cyanide decreases the oxygen consumption to a considerably greater extent than does ethyl urethane, although both produce the same degree of anæsthesia.

The literature dealing with the chemical, physiological and pharmacological properties of the cyanides and related substances has been extensively reviewed in the second paper of this series (Hyman, '19), and will therefore not be restated here. Briefly, it may be said that this group of substances has been shown to depress many physiological processes; and to decrease the rate of oxygen consumption or carbon dioxide output or both of the following living materials: yeast, a mould *Aspergillus*, a sponge *Suberites*, *Gonionemus*, *Planaria*, a beetle *Passalus cornutus*, embryos of *Fundulus*, several mammals, sea-urchin eggs, red blood corpuscles of geese, isolated mammalian kidneys, the frog's heart, minced beef liver, minced horse, beef, and pigeon muscle, and minced horse brain. In most of these cases it was shown that the effect was reversible. Since that review was written, two additional papers have come to my attention, those of Allen ('19) and of Evans ('19). Allen's results and conclusions regarding the effect of potassium cyanide on the rate of oxygen consumption of *Planaria* are about the same as my own, except for one or two points; thus Allen states that the oxygen consumption of *Planaria* cannot be reduced to less than 20 per cent. of the normal by cyanide, while I found in many cases, reduction to 10 to 15 per cent. of the normal. Evans's interesting paper contains some further references to the literature, confirms some previous work, and presents a number of new facts. Thus Evans finds that cyanide decreases markedly and reversibly the oxygen consumption of the cat, confirms the older statements that oxygen is just as readily dissociable from the blood in cyanide poisoning as from normal blood; and shows a striking similarity between the behavior of smooth, skeletal, and heart muscle, and nerve and nervous centers in the presence of cyanide and in lack of oxygen. Evans concludes that the cyanides "appear to exert all of their physiological effects by reason of the anoxæmia which they produce," and favors the view that they act by uniting chemically with some tissue constituent.[1]

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[1] Comment would seem to be required on Evans's remark (p. 23) that the observations of Lund and Herwerden seem to be incompatible with Child's conception of metabolic gradients. Lund's results have been considered elsewhere (Child, '19). Herwerden ('18) says that the buds and young of *Hydra* are not more susceptible to cyanide than the parents. Perusal of her paper shows, however, that she has compared only three or four pairs of individuals and has observed the *disintegration of the tentacles only*, having removed the cyanide when this occurred. Now, we have clearly shown (Child and Hyman, '19) that the buds of *Hydra* are not comparable with the parents *until they have the same degree of muscular activity*, and further that the susceptibility of the tentacles is also *dependent upon their degree of activity*. Miss Herwerden is mistaken in her conclusion. We have compared dozens of young and adult *Hydras*, belonging to three species, and have always found that the young are more susceptible to cyanide *when comparable degrees of muscular activity* exist. It is true that the difference is *least* in the case of the tentacles but even there the tentacles of the young are slightly more susceptible. Neither are Miss Herwerden's statements about the susceptibility of the Daphnid heart entirely correct. I have found that the smallest individuals in a *Daphnia* culture are the most susceptible of all (both as to heart and entire animal); that the susceptibility decreases with increasing size up through the size when the females are producing their first parthenogenetic broods; but from that time on, the relation is reversed, so that the largest and oldest individuals in the culture, which have produced many parthenogenetic broods, are more susceptible than

In the experiments to be reported in this paper it was not my purpose to make a complete study of the action of cyanide on oxygen consumption in these animals, since I had already done that in the case of a sponge and *Planaria*, but merely to show that cyanide in non-lethal doses reversibly decreases oxygen intake. The experiments on the marine forms were performed at the Marine Biological Laboratory, Woods Hole, Mass.; those on the fresh-water forms at the University of Chicago. I am greatly indebted to Professor F. R. Lillie for working space at the first-named institution.

The methods employed were identical with those used in previous experiments on this subject and will be found described in full in a former paper (Hyman, '19). Briefly, the animals to be tested were placed in Erlenmeyer flasks or wide-mouthed bottles of 500 to 600 c.c. capacity, these were filled air-tight with water, and the animals allowed to respire in them for a convenient length of time. A sample was then withdrawn, and this and a sample of the original water used were analyzed for oxygen content by Winkler's method, the difference between the two samples representing the oxygen consumed by the animals. Two separate determinations of the oxygen consumption in normal water were made, potassium cyanide was then added to the water and two more determinations in the presence of cyanide carried out. The animals were then washed in several changes of water, and their oxygen consumption in normal water tested again a day or two later. Throughout each experiment, the receptacles containing the animals were immersed in a water-bath, the temperature of which was kept constant to 0.5 degrees.

The possibility of iodine absorption by substances emanating from the organisms was again considered, and was tested in the case of two or three of the animals employed. Standard iodine solution was added to water which had been standing on the animals for at least one hour, and the iodine then titrated back

half-grown individuals. It would thus appear that in these animals parthenogenetic reproduction is accompanied by some degree of rejuvenescence. Green's recent work on *Simocephalus* (BIOL. BULL., Aug., 1919) supports this suggestion, since he found that these animals reproduce sexually early in ontogeny, and subsequently the same individuals begin parthenogenetic reproduction, a reversal of the order usually observed in parthenogenetic forms.

with thiosulphate. No loss of iodine was found, and indeed, this possible source of error in the Winkler method would seem to have been greatly overrated (*cf.* Hale and Melia, '13).

Experiments on Pennaria.

Pennaria tiarella, a common colonial hydroid of the Atlantic coast, was selected as a representative of this group of cœlenterates. Large quantities of freshly collected material were available. Clean branches, free from visible plant growth, were cut off, *Caprella* and other small animals which commonly live on such colonies carefully removed, and the branches were then placed in wide-mouthed bottles, and treated as described above. The animals were always used within a few hours after they were collected.

Table I.

Action of Potassium Cyanide on the Oxygen Consumption of *Pennaria tiarella*.

No. of Experiment	1.	2.	3.	4.	5.	6.
Temp. and Date	Aug. 18, 19° C.			Aug. 23, 22° C.		
Oxygen Consumed, Cubic Centimeters per Hour.						
First hour normal	0.55	0.56	0.43	0.94	0.78	1.21
Second hour normal	0.62	0.56	0.43	0.95	0.74	1.20
	1/25000 Mol. KNC.			1/10000 Mol. KNC.		
First hour KNC	0.38	0.38	0.27	0.52	0.45	0.60
Second hour KNC	0.32	0.35	0.25	0.36	0.34	0.54
Per cent. decrease	41	35	39	54	49	53

Six different lots of *Pennaria* were tested in this manner, as to their normal rate of oxygen consumption and the rate in 1/10000 and 1/25000 mol. potassium cyanide. The results are given in Table I. In all cases, the rate of oxygen consumption is reduced in the presence of cyanide. Unfortunately in the case of this animal it could not be determined whether the effect was reversible, since *Pennaria* deteriorates rapidly in the laboratory; within twenty-four hours, the majority of the hydranths have either fallen off or have lost their normal appearance.

Since *Pennaria* exhibits very few movements, it is not possible that differences in muscular activity could account for the decreased oxygen intake in the presence of cyanide. As far as could be determined, the hydranths were as fully expanded in the presence of cyanide as in normal sea-water.

EXPERIMENTS ON METRIDIUM.

Metridium marginatum was selected as a representative of the Anthozoa. The individuals used had probably been kept in float cars for some time. Medium-sized individuals were placed in wide-mouthed bottles of about 600 c.c. capacity, one in each, and left there undisturbed until they had attached themselves and expanded fully. It was found that the animals soon became accustomed to such an environment and could be handled and would submit to change of water without contracting. Since the oxygen consumption of sea-anemones in all probability varies with the degree of expansion, note was always made of the degree of expansion during the experiments. In experiments 1 and 3, the animals were slightly contracted during the exposure to cyanide but in the other six experiments they were fully expanded throughout. Differences in state of muscular activity are therefore not responsible for the observed results.

The eight experiments which were performed upon *Metridium* are reported in Table II. A marked and reversible decrease in oxygen consumption in the presence of cyanide was found in all cases.

TABLE II.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF *Metridium marginatum.*

No. of Experiment	1.	2.	3.	4.	5.	6.	7.	8.
Temp. and Date	Aug. 12, 21° C.			Aug. 10, 21.5° C.		Aug. 13, 20° C.		
Oxygen Consumed, Cubic Centimeters per Hour.								
First hour normal	1.33	1.19	1.13	1.66		1.06	1.01	0.97
Second hour normal	1.09	1.05	1.03	1.94	1.67	1.12	1.19	1.31
	1/5000 Mol. KNC.			1/10000 Mol.		1/25000 Mol.		
First hour KNC	0.48	0.56	0.56	1.24	1.25	0.81	0.70	0.66
Second hour KNC	0.49	0.59	0.42	0.89	0.70	0.70	0.76	0.59
Per cent. decrease	60	49	55	41	58	32	34	46
Recovery	1.09	1.10	1.14	1.68		1.08	1.18	1.20

Experiments on Polychætes.

Experiments were performed on three common polychæte annelids of the Atlantic coast—*Nereis virens*, *Arenicola cristata*, and *Chætopterus pergamentaceus*. Of the three species, *Chætopterus* was found to be the most favorable for this kind of experimentation, as it is not a very active animal. No difference in amount of activity in normal and cyanide-containing sea-water was observed. *Arenicola* was fairly favorable for the purpose as it also is relatively inactive, and here again there was no significant difference in activity throughout the experiments; indeed, it seemed to me that the respiratory movements were more pronounced in the presence of cyanide than in normal sea-water. On the other hand, the results with *Nereis* were not very satisfactory owing to the restlessness of the animals. In experiments 1–3, Table V, the animals were considerably more active in normal sea-water than they were after cyanide had been added, and hence the decrease observed in cyanide was due in part to diminished motor activity. In experiments 4–6, Table V., however, differences in motor activity were not present and have not contributed to the result.

A further difficulty was encountered in the case of *Nereis*. It was observed that both the degree of activity and the rate of oxygen consumption of *Nereis* diminished when it was kept in the laboratory. Experiments 1–3 were performed on freshly collected individuals and it will be noted that the oxygen consumption of these individuals is much higher than in the other cases, and further that the recovery figures, obtained upon them twenty-four hours later, are considerably lower than the original figures. This is not due to the exposure to cyanide since individuals from the same collection which had not been subjected to cyanide showed the same decrease in oxygen intake after a day in the laboratory. It is probable that this decrease is due in large part to starvation. I have observed it in the case of a number of other animals also, and my experience indicates that animals which have been kept for a few days after removal from their natural environment are preferable to freshly collected material for experiments of this kind. This difficulty was not encountered in the case of *Chætopterus* and *Arenicola* since it happened that the animals had been on hand in the collecting department for some time before I obtained them.

Since the first experiments showed that some little time was required for the penetration of even relatively concentrated solutions of cyanide into these animals, presumably because of their thick body walls, the practice of leaving them for an hour or two in the cyanide solution before carrying out the test was adopted.

TABLE III.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF *Chætopterus pergamentaceus.*

No. of Experiment	1.	2.	3.	4.	5.	6.	7.	8.	6.
Temp. and Date	Aug 3, 20.5° C.			Aug. 3, 21° C.			Aug. 5, 21° C.		
Oxygen Consumed, Cubic Centimeters per Hour.									
First hour normal	0.48	0.40	0.56	0.44	0.36	0.45	0.47	0.35	0.44
Second hour normal	0.33	0.40	0.48	0.40	0.36	0.44	0.49	0.32	0.43
	1/2000 Mol. KNC (Two Hrs. Before Test).			1/5000 Mol. KNC (Two Hrs. Before).			1/10000 Mol. KNC (One Hr. Before).		
First hour KNC	0.20	0.13	0.23	0.23	0.16	0.27	0.41	0.24	0.34
Second hour KNC	0.14	0.16	0.21	0.14	0.16	0.22	0.41	0.22	0.33
Per cent. decrease	59	64	58	56	56	45	15	32	23
Recovery	0.34	0.48	0.50	0.48	0.34	0.43			

TABLE IV.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF *Arenicola cristata.*

No. of Experiment	1.	2.	3.	4.	5.	6.
Temp. and Date	Aug. 2, 20.5° C.			Aug. 1, 21° C.		
Oxygen Consumed, Cubic Centimeters per Hour.						
First hour normal	0.42	0.39	0.67	0.42	0.48	0.43
Second hour normal	0.34	0.38	0.63	0.44	0.47	0.56
	1/1000 Mol. KNC (Two Hrs. Before).			1/2000 Mol. KNG (Two Hrs. Before).		
First hour KNC	0.27	0.30	0.46		0.22	0.31
Second hour KNC	0.20	0.26	0.46	0.27		0.30
Per cent. decrease	39	28	39	35	54	39
Recovery	0.49	0.31	0.71	0.42	0.48	0.43

The results with *Chætopterus* are presented in Table III., with *Arenicola* in Table IV, and with *Nereis* in Table V. One or two individuals, depending on size, were placed in each flask. For reasons already given, experiments 1–3, Table V., on *Nereis* were not very satisfactory but the other experiments on these polychætes leave no doubt that cyanide brings about a reversible decrease in oxygen consumption.

TABLE V.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF *Nereis virens.*

No. of Experiment	1.	2.	3.	4.	5.	6.
Temp. and Date	Aug. 5, 22° C.			Aug. 6, 21° C.		
Oxygen Consumed, Cubic Centimeters per Hour.						
First hour normal	0.80	0.65	0.65	0.30	0.36	0.29
Second hour normal	0.78	0.55	0.64	0.23	0.23	
	1/5000 Mol. KNC (Two Hrs. Before Test).			1/1000 Mol. KNC (One-half Hour Before).		
First hour KNC	0.14	0.11	0.11	0.05	0.05	0.08
Per cent decrease	83	82	83	83	84	73
Recovery	0.32[1]		0.48[1]	0.34		0.29

EXPERIMENTS ON LEECHES.

The leeches used in these experiments were *Hæmopis marmoratis*, and *Herpobdella punctata*, chiefly the latter. They were obtained from ditches near Wolf Lake, Indiana, and subjected to experiment shortly after they were brought into the laboratory. Two or three individuals were placed in each flask. As in the case of the polychætes, it was impossible to eliminate movement, but the degree of activity was about the same in both normal and cyanide-containing water. The results are given in Table VI. Experiments 4 and 7 were performed upon *Hæmopis*, the others on *Herpobdella*.

[1] Failure to recover original rate of oxygen consumption not due to cyanide, see text.

TABLE VI.

ACTION OF POTASSIUM CYANIDE ON THE OXYGEN CONSUMPTION OF LEECHES (*Hæmopis marmoratis*, EXPS. 4 AND 7, AND *Herpobdella punctata*, REMAINING EXPS.).

No. of Experiment	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Temp. and Date	April 3, 21° C.			Mar. 22, 21° C.			April 1, 21° C.			
Oxygen Consumed, Cubic Centimeters per Hour.										
First hour normal	0.15	0.22	0.19	0.51	0.31	0.28	0.51	0.21	0.24	0.20
Second hour normal	0.16	0.24		0.51	0.30	0.25	0.48			
	1/5000 Mol. KNC.			1/10000 Mol. KNC.			1/25000 Mol. KNC.			
First hour KNC	0.01	0.07	0.04	0.17	0.17	0.15	0.29	0.08	0.17	0.14
Second hour KNC	0.04	0.07	0.06	0.13	0.14	0.11	0.26	0.09	0.14	0.13
Per cent. decrease	84	70	74	73	51	51	45	60	36	33
Recovery	0.17	0.28	0.23		0.26	0.33		0.16	0.23	0.19

EXPERIMENTS ON OLIGOCHÆTES.

The forms used in these experiments were an aquatic earthworm, *Helodrilus tetædra*, one of the megadrilous oligochætes, and *Lumbriculus inconstans*, one of the microdrilous oligochætes. Both of these annelids live in temporary pools in the woods in the dune region of Indiana; the former occurs in the larger pools and also in permanent ponds, while the latter species has never been found in permanent bodies of water. They were collected near Clarke, Indiana. Both species are highly thigmotactic, being found in their natural habitat entwined among the branches of the moss which commonly grows in these pools or lying between layers of dead leaves, their posterior ends usually protruded for respiratory purposes. As the animals are very restless when their bodies are not in contact with objects, an attempt was made to quiet them by placing a small amount of thoroughly washed cotton in the experimental flasks with the worms for some hours preceding the tests. This procedure was entirely successful with *Helodrilus;* the worms entwined themselves among the cotton fibers and remained perfectly quiet throughout the experiments. It was possible to quiet the majority of the individuals of *Lumbriculus* in this way also, but a few individuals would always continue to crawl about. No difference, however, was observed in the degree of activity in normal and in cyanide-containing water.

The results with *Helodrilus* are presented in Table VII. and with *Lumbriculus* in Table VIII. As these worms are rather small, a large number of individuals was used in each experiment. The results are the same as in the case of the other animals tested, a reversible decrease in oxygen intake when cyanide is present.

Table VII.

Action of Potassium Cyanide on the Oxygen Consumption of *Helodrilus tetædra*.

No. of Experiment	1.	2.	3.	4.	5.	6.
Temp. and Date	June 3, 23° C.			June 2, 23° C.		
Oxygen Consumed, Cubic Centimeters in Two Hours.						
First hour normal	0.13	0.16	0.16	0.17	0.18	0.19
Second hour normal	0.13	0.19	0.15	0.16	0.22	0.22
	1/5000 Mol. KNC.			1/10000 Mol. KNC.		
First hour KNC	0.05	0.08	0.09	0.13	0.16	0.11
Second hour KNC	0.06	0.06	0.07	0.09	0.12	0.08
Per cent. decrease	59	60	49	30	30	54
Recovery	0.14	0.16		0.14	0.19	0.16

Table VIII.

Action of Potassium Cyanide on the Oxygen Consumption of *Lumbriculus inconstans*.

No. of Experiment	1.	2.	3.	4.	5.	6.
Temp. and Date	Oct. 17, 21° C.			Oct. 15, 21° C.		
Oxygen Consumed, Cubic Centimeters per Hour.						
First hour normal	0.44	0.47	0.37	0.58	0.58	0.46
Second hour normal	0.45	0.41	0.29(?)	0.57	0.61	0.49
	1/5000 Mol. KNC.			1/10000 Mol. KNC.		
First hour KNC	0.20	0.24	0.18	0.30	0.28	0.21
Second hour KNC	0.14	0.16	0.14	0.28	0.26	0.19
Per cent. decrease	62	55	52	50	55	56
Recovery	0.38	0.42	0.42			

SUMMARY AND CONCLUSIONS.

1. The normal oxygen consumption and the oxygen consumption in the presence of various concentrations of potassium cyanide was tested in the case of *Pennaria*, *Metridium*, *Nereis*, *Chætopterus*, *Arenicola*, two species of leeches, and two species of aquatic oligochætes. In all cases, numbering about seventy experiments (not all of which are reported), the oxygen consumption was markedly decreased in the presence of cyanide.

2. This decrease was not due to differences in muscular activity, since in some of the experiments it was possible to keep the animals entirely quiet throughout, while in the others, with three exceptions noted in the text, the animals were equally active in both the normal and the cyanide-containing water. None of the concentrations of cyanide used caused any visible anæsthesia, within the short time periods during which the animals were exposed to them.

3. The decrease was reversible, the oxygen consumption returning to approximately the original value when the cyanide was washed out of the animals. In no case were the animals injured in any way. Most of them were kept for a considerable length of time after the experiments were completed and were entirely normal in behavior and appearance.

4. As was found to be the case in previous experiments with cyanide, the percentage of decrease is absolutely greater, the more concentrated the cyanide solution, but the more dilute solutions are relatively more effective.

5. These results are in accord with previous experiments on the action of cyanides and justify the use of the cyanides as depressing agents.

LITERATURE.

Allen, G. D.

'19 Quantitative Studies on the Rate of Respiratory Metabolism in *Planaria*. I. The Effect of Potassium Cyanide on the Rate of Oxygen Consumption. Amer. Jour. Physiol., Vol. 48, pp. 93–121.

Child, C. M.

'19 A Comparative Study of Carbon Dioxide Production during Starvation in *Planaria*. Amer. Jour. Physiol., Vol. 48, pp. 231–257.

Child, C. M., and Hyman, L. H.

'19 The Axial Gradients in Hydrozoa. I. Hydra. BIOL. BULL., Vol. 36, pp. 183–223.

Evans, C. Lovatt.

'19 Observations on Cyanide Anoxæmia. Jour. of Physiol., Vol. 53, pp. 17–42.

Hale, F. E., and Melia T. W.

'13 Winkler's Method for the Determination of Oxygen in Water; the Effect of Nitrite and its Prevention. Jour. Indust. and Engineer. Chem., Vol. 5, pp. 976–980.

Herwerden, M. A. van.

'18 La résistance de cellules de divers âges a l'empoisonnement par le cyanure de potassium. Arch. neerl. de Physiol., Vol. 2, pp. 715–720.

Hyman, L. H.

'19 On the Action of Certain Substances on Oxygen Consumption. II. Action of Potassium Cyanide on *Planaria*. Am. Jour. Physiol., Vol. 48, pp. 340–371.

Loeb, J., and Wasteneys, H.

'13 Narkose und Sauerstoffverbrauch. Biochem. Zeitschr., Vol. 56, pp. 295–306.